THE SPERMATOGENESIS OF AGALENA NÆVIA.

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INTRODUCTION.

A study of the spermatogenesis of the Araneina offers a field of unusual interest to the investigator not only because of the seemingly aberrant form of the mature sperm cells but also because of the presence of conspicuous accessory chromosomes in many species. One needs only to read over the list of subjects in recent cytological literature to realize how much interest is at present centered upon the development of the germ cells as a whole and especially upon certain chromosomes which behave peculiarly and which are designated by various names — "chromosomes spéciaux" (de Sinéty), "Chromatin nuceolus" and "heterochromosome" (Montgomery), "small chromosome" (Paulmier), "accessory chromosome" (McClung), "idiochromosome," "macrochromosome" and "microchromosome" (Wilson). Nearly two decades ago, Henking ('90) in his work upon *Pyrrhocoris*, discovered and described a chromatin element which took part in only one of the spermatocytic divisions and therefore caused dimorphism of the spermatozoa but he did not apparently see the relation between this element and the chromatin nucleoli of the resting stage nor did he offer any suggestion as to its significance. Since the publication of Henking's work, scores of papers on insect spermatogenesis have appeared but as excellent reviews of this literature have already been given more than once, it seems superfluous to review it here. Suffice it to say that it is now a well-established fact that among the Hemiptera, at least, dimorphism of the spermatozoa is the rule and furthermore that the dimorphism is due to the unequal distribution of the heterochromosomes or to their being of unequal size. In the myriapods, also, Blackman ('01, '03, '05) has found the same dimorphism of the spermatozoa.

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Outside of the insects and myriapods, heterochromosomes have been reported in the Araneina only and as yet this unusually beautiful and interesting material has not only been comparatively neglected, but there has been marked discrepancy in the results of the few who have worked upon it. So far as I know, the only published work upon the development of the male germ cells of spiders which has been done under sufficiently modern methods of technique to be of value to us here is that of Wagner in 1896, myself in 1900, Montgomery, Bösenberg and myself in 1905 and Berry in 1906. With the hope of harmonizing the results of the above-mentioned authors and of correcting and expanding my own earlier results the present work was undertaken. It has seemed best to use the common tube-weaving spider, *Agalena nævia*, again as the basis of investigation, although a number of genera have been studied in order to illustrate some points. While my former conclusions in regard to the behavior of the ordinary chromosomes are now only verified and strengthened, those upon the distribution of the accessory chromosomes and upon the nature of the degenerating cells need considerable revision. After a careful reading of the literature on the subject and after an examination of testes in various genera, I am convinced that before long generalizations can be made in spider spermatogenesis and that contradictions are mainly due to differences in observations and interpretations; not to radical differences existing in the developmental history of the germ cells themselves.

My investigations were carried on in the Zoölogical Laboratory of the University of Pennsylvania and it gives me pleasure to warmly express my indebtedness to Professor Conklin for the kind interest with which he has followed the progress of my work and his helpful suggestions and encouragement.

Material.

Most of my material was collected in the state of Massachusetts where the breeding season of *Agalena* does not begin, usually, until the last week of August and continues until late in September. In mid-summer the testes are largely made up of primary spermatocytes in the growth period while in May and the

early part of June they consist mainly of spermatogonia. In Philadelphia the breeding season occurs a week or two earlier than it does in Massachusetts. Late in the fall the male spiders are fewer in number than the female spiders owing to the fact that they are more often overcome and eaten by their mates. Most of the adult spiders perish at the approach of cold weather.

The testes are translucent, slightly convoluted tubular organs and can be easily seen as they lie inbedded in the voluminous, brown liver which in mature specimens occupies the greater part of the abdominal cavity. In very young spiders, however, it is often difficult to distinguish the testes from the whitish, tubular spinning-glands lying beneath them. In *Agalena* it is a comparatively simple matter to determine the sequence of stages in the development of the germ cells as in cross-sections of the testes the least mature cells are always at the periphery, and they increase in maturity toward the lumen. Sometimes in a single cross-section can be found spermatogonia, spermatocytes, spermatids and mature spermatozoa. In the height of the breeding season the lumen and the ducts are filled with quantities of ripe spermatozoa and degenerating cells. As was first discovered by Menge ('43), the male spider has the peculiar habit of spinning a small, delicate web and depositing upon it a minute drop of seminal fluid which is then taken up into the fine, coiled tubes of the pedipalps preparatory to its introduction into the receptaculum seminis of the female. This process can readily be observed if spiders be kept in captivity during the breeding season and Montgomery ('03) has given a detailed description of it in a number of genera. By teasing out the contents of the storing-organs of the pedipalps one can obtain an abundance of spermatozoa which are sure to be mature.

Method.

The spiders were beheaded and the testes dissected out in the killing fluid. This method renders easy their removal from the body and insures rapidity of fixation. Among the various fixing fluids used were Zenker's fluid, Gilson's mercuro-nitric, Gilson's acetic alcohyl with sublimate, Hermann's fluid and Flemming's fluid, strong solution. Flemming's fluid gave slightly better results than Hermann's and both of these fluids gave vastly better

preparations than the others mentioned although all were useful in some ways.

Ripe spermatozoa from the pedipalps were smeared on a glass slide and well fixed by heating at the boiling point. Smear preparations, after the method of Foot and Strobell, proved of value for the mature sperm cells but were disappointing for the earlier stages. This may be due to the fact that the chromosomes are large and numerous and even when the nucleus is spread out in an extremely thin film, they become so heaped up upon one another that it is not possible to count them accurately. If smear preparations be compared with those treated with fixing fluids, it is evident that the latter cause considerable shrinkage; and tissues fixed in Flemming's fluid for a few hours show less shrinkage than those fixed for fifty hours or more but the latter method brings out strongly the centrosomes and spindle fibers.

Heidenhain's iron-hæmatoxylin has been chiefly relied upon for staining as it gave the finest results in almost every particular but beautiful preparations were also obtained with Hermann's triple stain. Every one grants that the different reactions to staining reagents are no safe criterion in the endeavor to differentiate nuclear elements, since the same structures do not always stain in the same way during the various phases of development. Nevertheless color-differentiation is often helpful and this is especially true when dealing with the accessory chromosomes. Their whole history can be made out clearly in iron-hæmatoxylin preparations but when Hermann's triple stain is used, their affinity for the safranin at times when the ordinary chromosomes or the chromatin granules take the violet, makes them stand out in a striking manner.

Observations.

Spermatogonia.

In young spiders, early in the summer, the testes are wholly made up of spermatogonia and, unlike most forms, these cells in mitosis are almost equal in size to the spermatocytes of the growth period. In the metaphase the rod-shaped chromosomes are so numerous, probably fifty or more, and so closely packed together, that in polar views it is impossible to make even an approxi-

mately accurate count of them (Figs. 1 and 2). It was also not found possible to identify at this stage the accessory chromosomes among the other rod-shaped chromosomes. The centrosomes are quite distinct and the spindle fibers have a tendency to bulge out, forming a spindle with convex sides. This is probably owing to the large size of the chromosomes and to the large number of fibers which must be accommodated between the centrosomes and the equatorial plate. In division the rods split longitudinally and the resultant halves move toward the opposite poles. In anaphase two pairs of daughter chromosomes appear distinct from the others both on account of their large size and because they are slower in passing to the poles (Figs. 3 and 4). While this might well be looked upon as simply a case of retarded division, in the light of what follows it seems probable that they are the accessory chromosomes which have the form of a pair of rods throughout the greater part of their subsequent history. In telophase the ordinary chromosomes gradually become granular but their identity is still traceable in the loose, irregular masses scattered through the nuclear cavity. These masses stain but faintly, and in iron-hæmatoxylin preparations, when the extraction of the dye has not been carried further than usual, they are nearly colorless. The accessory chromosomes, on the other hand, retain the rod-like form and lie near the periphery of the nucleus.

Spermatocytes.

In Fig. 5 is shown the early prophase of the primary spermatocyte in which the chromatin masses are becoming looser, more granular, until finally the processes of adjacent ones meet on the threads of the linin reticulum. The nucleus swells with the accumulation of nuclear sap and at the same time the cytoplasm is reduced to a rather thin layer around the swollen nucleus. The accessory chromosomes lose their rod-like form and appear as two densely staining, chromatin nucleoli often lying at some distance apart (Fig. 6). Later these two chromatin nucleoli approach one another and finally unite to form a single, large, irregular mass which is a conspicuous body in the resting stage (Fig. 7). This is followed by the contraction stage when the whole cell is noticeably reduced in size and the nuclear contents

are contracted away from the membrane. At this time the nucleus stains so deeply that only after long extraction can anything be learned of its structure and even then one can merely say that the chromatin appears to be in the form of a dense reticulum. It is apparently at this stage that synapsis occurs, reducing the number of chromosomes to half the spermatogonial number. The large, chromatin nucleolus of the resting stage has again resolved itself into two peripherally placed rods shown in longitudinal and cross sections (Figs. 8 and 9). At the close of the contraction stage the growth period is introduced by an evident increase in the size of the cell and the nucleus. As the nuclear elements become spread out in the enlarging cavity it is comparatively easy to see in what condition they are. The chromatin is now in the form of irregular granules distributed along the delicate loops of the spireme. The loops are long and often twisted or bent, so that their number was not determined nor could I ascertain whether or not the spireme is continuous but taking what evidence one can gather from this stage, together with a study of a slightly later stage, it seems probable that the loops are distinct from each other and that the segments of the spireme are in the reduced number (Figs. 10 and 11). Later the nucleus enlarges still more, allowing the loops to stretch out fully and it is now clear that the spireme is segmented, the free ends of the loops all being directed toward one side of the nucleus (Fig. 12). The linin thread is delicate, at first, and the chromomeres undivided, but later a split runs throughout the length of each loop, dividing the chromomeres equally. Sometimes the latter appear as large after the splitting as before but this is readily explained when one considers that meanwhile the loops have shortened and so have crowded the chromomeres into larger groups. At first glance the accessory chromosomes, on account of their great staining capacity, appear to have maintained their peripheral position but careful focusing reveals the fact that they are now completely surrounded by the spireme loops (Fig. 13). A cross-section of the same stage is shown in Fig. 14. Although the accessory chromosomes have moved to a more central position in the nuclear cavity, their outer ends are still near the membrane and are

always directed toward that part of the cell which contains the greatest amount of cytoplasm and in which the centrosome lies. This pole has been called by Montgomery the distal pole. The closed ends of the spireme loops are directed toward the opposite or central pole. The same relative position of the loops, the accessory chromosomes and the centrosome is retained throughout the growth period and gives striking evidence of cell polarity. At a slightly later stage the accessory chromosomes seem to be simulating the structure of the spireme to a limited degree. They temporarily lose their compact form, become distinctly granular and extend in length over about two thirds of the diameter of the nucleus (Fig. 15). Gradually they shorten again and conjugate side by side, the union usually first taking place at the end directed toward the central pole and progressing toward the distal pole (Fig. 16). Cases are found in which the union begins simultaneously at the two extremities, giving a ring-like form. After the union is completed they appear as a single mass when viewed either in longitudinal or cross section (Figs. 17 and 18). Whether in this conjugation a complete fusion of the two elements occurs or whether it is merely a close approximation, I am unable to say, but soon the single mass is again split into the two characteristic rods.

When the longitudinal split in the spireme has reached its widest extent, some of the loops still extend nearly across the nuclear cavity but later they begin to draw down toward the distal pole. As the shortening continues, the longitudinal split becomes less and less evident but indications of it can be detected at a late stage. Judging also from the subsequent history I believe that the split persists, being merely lost sight of in the close approximation of the two moieties during the process of contraction (Figs. 19–21). In the late prophase, the loops of the spireme not only shorten but bend to an acute angle to form V-shaped chromosomes which then open out into double V's (Fig. 22). The chromatin of these is in a more or less granular condition and leaving their former position at the distal pole they are distributed through the nuclear cavity. The rod-like accessory chromosomes remain unchanged both in form and in position. Soon the nuclear membrane disappears, the ordinary chromo-

somes, having reached their definitive form, become more compact and densely staining and spindle fibers appear (Fig. 23). In metaphase all of the double V's are drawn into the equatorial plate and the plane of division passes through the center of each, giving rise to single V's which are carried to the opposite poles. The accessory chromosomes lie at the periphery of the equatorial plate and are connected by spindle fibers to one pole only, a single fiber passing from the centrosome to each of the two rods. In the succeeding division they pass into but one of the two daughter cells, so that we find half of the secondary spermatocytes with accessory chromosomes and half without them (Figs. 24–26). Those containing the accessory chromosomes might be called "favored cells" ("bevorzugten zellen") as suggested by Henking. In polar views of the telophase several sections were found in which each of the two accessory chromosomes showed a distinct longitudinal split — a precocious splitting which is of interest as foreshadowing the division which occurs in the following mitosis (Fig. 27).

While the arms of the V-shaped chromosomes are elongating and becoming sinuous or twisted in outline, a conspicuous cell-plate forms and the constriction of the cell body grows deeper (Fig. 28). Since the chromosomes are long and twisted at this stage it is plain that even in fairly thin sections there might occur more than one section of a single chromosome and therefore little dependence could be placed upon the number counted in successive sections of the same nucleus. Figures 29 and 30 show the next stage with the accessory chromosomes in longitudinal and cross section respectively. The nuclear membrane has formed, the chromosomes are resolved into granules distributed on the nuclear reticulum and complete division of the cell body gives rise to two daughter cells, the secondary spermatocytes. Even at a very late stage of this process remains of the interzonal fibers, with the cell-plate, are conspicuous. In the late prophase of the secondary spermatocytes there arises from the preceding resting-stage a number of slender, twisted chromosomes closely resembling those which entered the resting stage and not infrequently the accessory chromosomes show a precocious, longitudinal split (Fig. 31). As the ordinary chromosomes are drawn into the

equatorial plate prior to the second maturation division, their V-shape becomes evident. The apex of the V is the point of attachment of the spindle fibers, and the free, sinuous arms extend in various directions away from the spindle axis, giving a bushy appearance to the mitotic figure (Fig. 32).

On account of this arrangement of the ordinary chromosomes it is now extremely difficult to identify the accessory chromosomes. Also the fact that they are present in only half of the secondary spermatocytes lessens the chances of finding sections cut in a favorable plane for their identification. In spite of these disadvantages they can in some cases be clearly seen at the equator of the spindle where they lie near together and at right angles to the spindle axis. Sometimes indications of the longitudinal split can be detected (Fig. 33).

The arms of the V-shaped chromosomes shorten and thicken while they also become straight and densely staining. The plane of division passes through the apex of the V's and the rod-like arms move to the opposite poles. The accessory chromosomes divide along the line of the longitudinal split and their resultant halves pass to the opposite poles a little more slowly than the ordinary chromosomes. They are also distinguishable by their larger size. It is now apparent that one half of the spermatids will be "favored cells," containing two accessory chromosomes, while the other half will not be favored (Figs. 34–39). In polar views of the anaphase attempts were made to determine the chromosomal number but after the utmost care I can give only the probable number. In the majority of cases twenty-five chromosomes were counted (Fig. 37), and their straight, rod-like form makes it improbable that any of them were counted twice. Occasionally twenty-four, twenty-six or twenty-seven were counted, all of them appearing to be ordinary chromosomes. It seems now as if the reduced number must be at least twenty-five, instead of nineteen as given in my previous paper. In telophase, before the nuclear membrane forms, the ordinary chromosomes again become slightly sinuous in outline. The daughter cells must often move through a considerable arc in the process of separating, as sections are found showing two cells not yet completely seperated and at the same time showing approximately

polar views of their respective chromosomes all in one plane. The accessory chromosomes appear thick and heavy at this stage (Figs. 40 and 41). Later the ordinary chromosomes are lost to view in the chromatin reticulum and give rise to the resting nuclei of the spermatids.

Transformation of the Spermatids.

One marked characteristic of the spermatids is that complete separation of sister cells is long deferred and in the early stages the cell-plate and interzonal fibers are conspicuous in the cytoplasmic neck connecting the two cells (Figs. 42 and 43). Near the cell periphery lies the centrosome from which an extracellular axial filament has grown out and this filament bears at the center and at the tip a transparent vesicle which stains deeply in iron-hæmatoxylin. The accessory chromosomes always lie near the distal pole of the nucleus with their outer ends turned toward the centrosome, so that here again we have the cell-polarity as beautifully shown as it is in the spireme stage. This ability to orient the cell brings to light the fact, already referred to, that sister spermatids must often undergo considerable rotation when they are drawing apart. In Figs. 44 and 45 the interzonal fibers have apparently disappeared but in all probability they give rise to the idiozome as claimed by Bösenberg ('05). At the center of the cytoplasmic neck connecting sister spermatids there is frequently a more or less evident enlargement which is of sufficiently general occurrence to deserve mention and in its center one finds the persistent mid-body (Zwischenkörper) even at a late stage (Figs. 46–51). This enlarged portion of the neck was called by Wagner the "connecting-body."

The nucleus now takes a form which bears a strong resemblance to the contraction phase of the growth period and were it not that it is of general occurrence in beautifully fixed material, it might be thought due to the harmful action of the fixing fluid. The chromatin reticulum contracts toward the distal pole of the nucleus into a mass which stains intensely, while the greater part of the nuclear cavity is left empty or is filled with nuclear sap. The accessory chromosomes lie in a distinct vesicle or at least in a clear space which gives them prominence and although they

are closely pressed together the double nature of this nucleolus-like mass is easily demonstrated after long extraction in iron-alum (Figs. 46 and 47). As to what happens during this process of contraction I am wholly in the dark but later the nuclear cavity is fully occupied by a delicate reticulum upon which the chromatin granules are distributed in such a finely divided condition that they show very slight affinity for staining reagents (Fig. 48). The centrosome has divided into a proximal and distal portion and the proximal centrosome has moved some distance over the nucleus or has possibly entered into its interior. During its passage it gives rise to an intra-cellular filament which connects the proximal and distal centrosomes. The extra-cellular axial filament is now larger and its vesicles have increased noticeably in size. The accessory chromosomes are no longer inclosed in a vesicle but unite side by side into a single, elongated rod which leaves its former position at the distal pole and travels to the central pole in a line nearly or quite parallel with the cell-axis (Fig. 49). Following this stage the nucleus changes in outline, becoming somewhat pear-shaped and the proximal centrosome, which has become large and irregular in form, has passed over about one half of the length of the nucleus. The chromatin shows a tendency to collect at one side of the nucleus to form the chromatin plate. In half of the spermatids the fused or nearly fused accessory chromosomes occupy the center of the chromatin plate, extending from the central to the distal pole of the nucleus, or, in other words, from the anterior to the posterior end of the rapidly forming spermatozoön head (Figs. 50 and 51). The chromatin plate increases in size until all of the chromatin reticulum is involved; the whole nucleus becomes much longer than broad with the extremities slightly curved. The sister spermatids now separate completely, the rupture occurring on each side of the "connecting-body" when it is present. Figure 53 shows a somewhat later stage where the transformation is complete. The pear-shaped nucleus of the spermatid has been transformed into the crescent-shaped head of the spermatozoön and the chromatin is so compact that the whole head has a dark, grayish hue after long extraction in iron-alum. Even at this late stage the

dimorphism of the spermatozoa is not concealed, for in half of them can be seen a slender, darkly stained band extending along the middle of the convex surface of the head, from the anterior to the posterior end, although it fades out often near the hinder extremity. This chromatic band represents the fused and somewhat modified accessory chromosomes whose distribution to but one half of the spermatozoa divides them into two distinct groups (Figs. 53 and 54).

In the mature spermatozoön the distal centrosome is no more in evidence, the axial filament has increased in length and its vesicles have disappeared. Whether or not the vesicles contribute their substance to the axial filament as it grows in length and whether or not the latter is supplied with a cytoplasmic investment, I am unable to say. The proximal centrosome forms a slight projection on the lower side of the head and probably corresponds to the end-knob of other forms. Wagner described it as a "little tooth" which lies at the point where the axial filament joins the chromatin plate and Bösenberg regards it as the middle piece, or rather as the "connecting piece," the former term not being applicable in the case of the spider spermatozoön. At the anterior end of the head is a transparent, apparently cylindrical body which in *Lycosa*, according to Bösenberg, is derived from the idiozome vesicle. Forming an axis in this apical body is a distinct fiber or filament which projects beyond the apical body and bears a deeply staining granule at its extremity. I cannot state positively the origin of the apical granule or of the filament which bears it but in some preparations, after long extraction, there is seen what appears to be a delicate filament passing from the end-knob, through the anterior portion of the head, and becoming continuous with the filament in the apical body. The distinctness of the filament within the head is exaggerated in the figures.

The spermatozoön now works itself free from the cell-body, the anterior end of the head protruding first. The escape is probably effected by the contractions of the head itself. Even after the posterior end of the head has entirely lost its connection with the cell-body, the spermatozoön is not yet ready to pass into the lumen of the testis (Fig. 55). First there occurs a very

perceptible decrease in size through a closer and closer crowding of the chromatin granules which compose the head and through contraction of the nuclear membrane which incloses them. As the contraction progresses the staining capacity diminishes, in iron-hæmatoxylin preparations, and the spermatozoön head has a solid, grayish appearance. The accessory chromosomes, however, can still be recognized in the purplish band on the convex side of the head. In the second place, when reduction in size is at an end, the interesting process of rolling or coiling begins. The anterior and posterior ends of the head bend toward one another until they overlap to form a ring-like or disk-like body which well conceals the actual structure (Fig. 56). During the rolling up process Wagner believed that the tail coiled itself into a little, matted clump near the "tooth" (end-knob) and was therefore finally inclosed at the center of the ring when the rolling of the head was completed. Bösenberg, on the other hand, thinks that by careful focusing he can detect the tail wrapped around the outer circumference of the ring. My own observations lead me to agree with Wagner on this point for in partially coiled spermatozoa I have seen an extremely small, darkly stained mass which apparently depends from the end-knob and I have seen no evidence of a tail wrapped around the outer circumference. Since both Wagner's work and my own has been done chiefly on *Agalena* and Bösenberg's descriptions mainly refer to *Lycosa*, it is quite possible that both opinions are correct and that the method of disposing of the tail differs in the two genera. When all of the spermatozoa in a given cyst have almost or altogether completed the process of coiling, the cyst-wall ruptures and allows them to escape into the lumen of the testis and later into the sperm ducts. Even after they have been stored in the pedipalps they remain coiled but can be forced to uncoil if spread on a glass slide and heated to the boiling point.

If the spermatozoa in the proximal portion of the ducts be compared with those which have passed into the more distal portions, or with those in the pedipalps, there will be noticed a marked difference in their appearance. Instead of retaining the ring-like form, they become decidedly longer than broad and in fixed material the chromatin shows a tendency to shrink away

from the outer wall leaving a clear area between it and the chromatin mass. Wagner described the final form of the spermatozoön as being rod-like and was criticized in this by Bösenberg who suggested that he was misled by seeing cross-sections of the disk-like sperm. This cannot possibly be the explanation for not only are all of the spermatozoa in the distal portion of the ducts and in the pedipalps in this elongated form but they are larger than when in the proximal portion of the ducts or in the lumen. This fact precludes the possibility of their being cross-sections (Fig. 59). Although they remain coiled they seem to lose the compact structure, which they assume before leaving the cysts, and regain their original size. This reëxpansion of the heads seem so improbable that I was only convinced of its truth after making numerous camera lucida drawings with utmost care. The difference in size and form can be seen at a glance by comparing Figs. 56 and 59 which were drawn with the same magnification. In all probability the spermatozoa first uncoil after they are passed through the slender ducts of the receptaculum seminis into the oviduct of the female spider.

When one considers that the tail is attached to the lower, anterior margin of the relatively heavy, crescent-shaped head, it is difficult to see how its lashing movements would propel the head in a straightforward direction. Bösenberg has made considerable study of the movements of spider spermatozoa and he holds that the chief propelling power is found in the twisting and bending motions of the head itself while the tail, by its lashing movements, may act as a steering-organ.

Sequence of Divisions.

In the maturation of the male germ cells it is generally conceded that two kinds of division occur — a reductional and an equational one — but in regard to their sequence there has been much difference of opinion. At a time when the majority favored the view that the equational division occurs first, Montgomery ('04, '05) maintained that the first division is reductional and he strongly emphasized the importance of determining the *origin* of the chromosomes in synapsis as their mere form in post-synapsis stages is often misleading. To-day perhaps the majority of

workers can be said to favor Montgomery's view that the primary spermatocytic division results in a separation of maternal and paternal chromosomes which have conjugated in the synapsis stage. In my previous work I pointed out that *Agalena* offers especially favorable material for the investigation of this point and also that my results led to a full endorsement of Montgomery's interpretation. Further study has convinced me that in all probability that view is the correct one. Every one who has tried to follow the behavior of chromosomes during synapsis knows the difficulties in the way of reaching any certainty about the matter. What we do know is that, as a rule, before synapsis we find a certain number of chromosomes present and after synapsis we find but half that number. Many facts can be advanced in support of the theory of the conjugation of maternal and paternal chromosomes resulting in numerical reduction and if such a pairing of these nuclear elements does occur it may be brought about either by an end to end union of homologous chromosomes or by a union side by side. It is clearly of utmost importance to determine which method of union obtains before endeavoring to interpret what follows.

In *Agalena*, in the early prophase of the primary spermatocytes, the nucleus becomes contracted and its structure cannot be fully made out, but the chromatin seems to be in the form of a dense reticulum. At this time I believe the synapsis to occur both because there is no later stage in which there is any sign of its occurrence and also because the delicate spireme loops which issue from this stage appear to be in the reduced number. Strong evidence against the view that a side to side pairing of the chromosomes occurs is the fact that the spireme at first shows no trace of a split, then a barely perceptible one and finally quite a wide one running the whole length of each loop. The space intervening between two threads, therefore, must represent a longitudinal split and not the space between two threads about to unite side by side.

As evidence in support of the view that the chromosomes unite end to end, I would call attention to the noticeably denser structure of the chromatin at the bend of the loops which is quite marked in some stages, the chromomeres appearing to be massed

together at this point. In iron-hæmatoxylin preparations the bend of the loops takes a deeper stain than the other portions do, while in safranin and gentian violet preparations the chromomeres at the bend of the loops take the safranin and the arms of the loops stain violet. Since condensed chromatin always does take the safranin, this differential staining may indicate merely a compact structure due to the mechanical bending of the loop but it may also indicate the junction of homologous chromosomes. In the light of the above facts, I consider it highly probable, therefore, that the split in the spireme represents a precocious longitudinal division which is more or less visible throughout the prophase of the first maturation division.

The succeeding steps are as follows: The longitudinally split loops become shorter and consequently thicker, drawing down toward the distal pole. The bend becomes acute, forming V-shaped chromosomes which split from apex to base along the line of the original longitudinal split and open out into double V's. At this point the chromosomes can be easily oriented for the split extends entirely through the free extremities of the arms of the V while the apices show the compact structure characteristic of the bend of the spireme loop. When they have taken up their final position at the equator of the spindle, one has no trouble in determining with certainty that the angles which correspond to the bend of the loop lie in the plane of division, while the angles corresponding to the free ends of the loop are directed to the opposite poles. Still further assurance, if needed, is gathered from the fact that sometimes a chromosome, which has not yet opened out, is drawn into the equator of the spindle and there always lies with its apex in the equatorial plane and its free ends directed toward the poles. Later this single V opens out into a double V preparatory to division. The first division then takes place through what corresponds to the apex of the original V-shaped chromosome and if this represents the point of union between the homologous elements, the first division must be reductional.

In the V-shaped chromosomes of the telophase, the space between the two arms corresponds to the split which first appears precociously in the spireme. When, in the succeeding division,

the two arms are carried to opposite poles of the spindle, we plainly have a longitudinal or equational division. It is also of interest here that in this mitosis the rod-like accessory chromosomes divide equationally. To sum up, then, the first maturation division occurs at a point corresponding to the bend of the spireme loop and is a reductional one; the second maturation division passes along the line of the longitudinal split of the spireme loop and is an equational one.

Individuality of the Chromosomes.

Every living organism, whether plant or animal, single-celled or many-celled, is regarded as an individual notwithstanding the fact that each one is so lacking in stability that, in its metabolic processes, it has frequently been compared to a whirlpool into which and out of which new particles are constantly streaming. Again, in the ontogeny of a Metazoan, the cells of which it is composed and the cell-nuclei are supposed to be continuous from one cell generation to the next. When, however, we come down to one of the most important nuclear elements the chromosome, there has been much difference of opinion in regard to individuality, some claiming that when a given chromosome disintegrates and spreads out in a reticulum in the resting stage, the same chromosome does not reappear in the succeeding mitosis but that the chromosomes are formed anew each time. Ever since Rabl ('85) strongly supported by Boveri ('87, '88) and Van Beneden ('83) maintained that the chromosomes do not lose their individuality at the close of division but persist in the chromatic reticulum of the resting nucleus, scores of workers have brought forward evidence either for or against this theory. In a recent paper by Foot and Strobell ('07 (*b*)) we read as follows: If we mean by "Individuality of the Chromosomes" merely that we recognize certain characteristics of size and form in some of the chromatin units called chromosomes and that there is a frequent repetition of these forms during different stages of development, then we may claim that the chromosomes of *Anasa tristis* unqualifiedly support the theory of the "Individuality of the Chromosomes." But on the other hand, if by "Individuality of the Chromosomes" we claim their morphological continuity, that several or even

only one of the chromosomes can be followed *uninterruptedly* from the spermatogonium to the spermatid, that even during the growth period the chromosome form is maintained, then we must say that in our preparations *Anasa tristis* supports in a very restricted sense, if at all, the theory of the "Individuality of the Chromosomes."

While all agree that the term individuality should not usually be taken in its narrowest sense in reference to chromosomes, the general fact that the same number of chromosomes issues from a reticulum as passes into it and that they have been seen to reappear in the same positions within the nucleus, added to strong evidence found in studies of fertilization of the egg of *Ascaris* and other forms, seem to me to clearly indicate genetic connection between the chromosomes in successive cell-generations. Even if the chromosomes do resolve themselves into their component granules which are distributed on a linin reticulum, it is not difficult to conceive of each one thus spreading out along definite lines, its ultimate branches temporarily anastomosing with those of adjacent ones, as a method of interchange of material or as a method of gaining nutriment for each granule which would be much less easily done in the dense, compact form. The probability is that the so-called resting stage is a stage in which physiological activity of the chromosomes is at its height. An *Amœba*, whether it be in an encysted form or whether it be spread out into a protoplasmic mass of extreme delicacy, with numerous pseudopodia, is still an individual *Amœba*.

In *Agalena*, the ordinary chromosomes offer no strong evidence in favor of chromosomal continuity although the loops of the spireme differ in length and in the prophase of the primary spermatocytes some of the V-shaped chromosomes differ slightly in size. One of these which opens at a much wider angle than the others recurs again and again, and is probably always present at this stage. When we turn to the accessory chromosomes, on the other hand, we find that they stand a more severe test than that outlined above and even comply with the demands of Foot and Strobell when they claim that, to meet the requirements for individuality, several or only one of the chromosomes should be followed uninterruptedly from the spermatogonium to the sper-

matid and that even during the growth period the chromosome form should be maintained. Not "only one" but *two* accessory chromosomes described in this paper have been followed without loss of identity from the spermatogonium through the growth stage, prophase, metaphase, telophase down to the spermatid and they more than meet the above requirements in that they have been traced to their final position in the head of the spermatozoön. The fact that they become granular and partially disintegrate for a short time in the growth period only shows that the granules, of which they are composed, separate from each other as in the other chromosomes but to a much less degree (Fig. 15). There is every probability that the other chromosomes have genetic continuity just as truly as the accessory chromosomes have it but at certain definite periods, possibly of great physiological activity, they take a form which temporarily obscures their individuality.

Degenerating Cells.

In my earliest studies upon *Agalena* I noticed many cases in which the mature spermatozoa seemed to be escaping from their respective cell-bodies and I then supposed that the latter could take no part in the formation of the germ-cells, but that they passed with them into the ducts and served as nutriment. Later, upon examination of some preparations made from another spider, *Pholcus phalangioides*, two kinds of degenerating cells were found in the lumen of the testis, one kind being supplied with brilliantly stained nuclei, while the other kind appeared granular and non-nucleated. These two kinds of cells were looked upon as early and late stages in the process of degeneration and the presence of chromatin precluded the possibility of their having originated from the cell-bodies discarded by the spermatozoa. The occurrence of spermatozoa wholly or partly free from their cell bodies was thus explained as a mechanical effect of sectioning with a microtome knife as it seemed likely that compact, resistant bodies like the sperm-cells might thus happen to be dislodged from the soft, protoplasmic mass in which they lie. Whence, then, came the great number of degenerating cells in the lumen of the testis and in the ducts? An answer to

this question was sought in the unequal distribution of the accessory chromosomes. They were found to take no part in the first maturation division and, while their further history was extremely difficult to follow, I gathered, as I then believed, some evidence of their taking no part in the second maturation division, and therefore of their final distribution to but one fourth of the spermatozoa. McClung's theory that the accessory chromosomes might be sex-determinants was then held to be untenable, so far as its application to the spider was concerned, as it did not seem probable that one sex would be three times as numerous as the other. I then ventured to suggest a new theory viz.: that only the one fourth of the spermatozoa which contain the accessory chromosomes — the "favored" spermatozoa — become functional while the remaining three fourths degenerate after almost or altogether reaching maturity. There were *a priori* reasons for believing such to be the case since, if true, the parallelism between the spermatogenesis and the oögenesis would be even more complete than hitherto supposed, three of every group of four daughter cells descended from a single spermatogonial cell being considered as homologues of the polar bodies which do not become functional. I also suggested, in view of the foreseen difficulties in the union of the sex-cells, that in the maturation of the egg the accessory chromosomes might be thrown off in the polar bodies and thus, at time of fertilization of the egg the normal chromosomal number would be restored. This second suggestion was overlooked by Boring ('07) in her criticism that if only the "favored" spermatozoa become functional, the egg must necessarily contain the accessory chromosomes also and that in the nucleus of the fertilized egg the chromosomal number would exceed the normal number by two. Berry ('06) in her paper on *Epeira* states that certainly in none of her preparations does she find any trace of degenerating spermatozoa, and other writers have expressed doubt of their existence. Now, while I still find an abundance of degenerating cells in the lumen of the testis and in the sperm ducts, my recent investigations have convinced me of the error of my former results in regard to the distribution of the accessory chromosomes. In the present paper I think it is clearly demonstrated beyond the shadow of a doubt that dimorphism of spermatozoa is the rule.

The above-mentioned facts have necessitated a careful re-examination of the whole subject of degenerating cells in the spider testis and after a brief review of the work of other writers, I shall give my present interpretation in the light of recent study upon this point.

Many of the earlier workers upon spider spermatogenesis have observed and described numerous, granular, protoplasmic bodies in the lumen of the testis, in the sperm ducts and pedipalps and also in the receptaculum seminis of the female spider. Most of them agree that these granules or granular masses have a nutritive function but they account for their origin in various ways. In *Tegeneria*, Bertkau ('77) believed that they arose from certain granular cells of the testis and sperm-ducts. Schimkewitch ('84) describes two kinds of cells in the testis of *Epeira*. Those at the posterior end, according to him, develop into spermatozoa. Those at the anterior end and also cells of the sperm ducts give rise to roundish or oval granules. In *Lycosa*, Birula ('94) found granular masses which were derived from the fragmentation of some of the follicle cells. On the other hand, Balbiani ('97) arrived at the conclusion that the spider testis is supplied with gland cells which pour out a secretion in the form of little granules. Wagner ('96*a*) maintained that the remains of the spindle fibers and Zwischenkörper fragment to form the "granules séminaux." Later appeared Bösenberg's paper ('05) in which I find statements which exactly accord with my earliest conclusions that the ripe sperm-cell works its way out of the cell-body and that the latter is left to degenerate.

"In der letzten Phasen der Umformung der Spermatiden in das Spermatozoön wies ich nach, dass der Kopf des Spermatozoöns mit dem Schwanz das Cytoplasma verlässt, welches dann in Form von grossen, runden Ballen im Follikel zurückbleibt. Diese Plasmakugeln degenerieren und zerfallen in kleine, runde Körnchen." Also, according to this author, the cells of the cyst-walls and their nuclei degenerate, the fragments of which pass into the lumen of the testis after the escape of the spermatozoa from the cysts. This whole mass of degenerating cells which completely envelop the rolled up spermatozoa in the ducts and pedipalps, Bösenberg regards as a possible source of nutriment

for the sperm-cells until they reach the ova at the time of fertilization.

In my first study of *Pholcus phalangiodes*, already referred to, it will be remembered that two kinds of degenerating cells were found in the lumen of the testis, half containing nuclei and half not. I have now found that these bear no genetic relation to each other but arise in totally different ways. Those without nuclei are the cytoplasmic remains discarded by the spermatozoa and which accompany the latter when they pass into the lumen of the testis. Those containing distinct nuclei originate from degenerating spermatids, many cases occurring where nearly all or quite all of the spermatids in a cyst are in advanced stages of degeneration. The cell-body becomes enlarged and vacuolated and the chromatin forms homogeneous-looking masses irregular in outline or fragmented (Fig. 60). These cells greatly decrease in circumference, the chromatin mass becomes spherical, and the cell body becomes so transparent that it is easy to overlook it altogether. They vary considerably in size, even after they have passed into the lumen of the testis but they are readily distinguished from the ripe spermatozoa and the granular cytoplasmic bodies among which they lie (Fig. 61).

In *Agalena*, although in the breeding season the sperm ducts are fairly packed with nearly colorless cells or fragments of cells in which the ripe spermatozoa lie embedded, none of the former appear to contain chromatin, or, if they do, it is so finely distributed that it stains very faintly. Neither in this spider have I found cysts full of degenerating spermatids. After a careful study of the cysts containing nearly mature or mature spermatozoa, I am thoroughly convinced that Bösenberg is correct in stating that the ripe sperm-cells wriggle out of the cell body and furthermore he claims to have actually witnessed the process in his examination of living cells. I have, in fixed material, found the sperm-cells in all stages of the process and the phenomenon is of too common and too general occurrence to be accounted for by the tearing action of the microtome-knife in sectioning. After the spermatozoa have wriggled themselves free, they remain in the cyst for some time before the rupture of its walls and during this time, while they are contracting and rolling up into a

disk-like form, described before, the cell-bodies from which they have escaped, also gradually contract, the circumference becomes greatly reduced and the density of the cytoplasm gives it a purplish hue in iron-hæmatoxylin (Fig. 58, *a–e*). When the cyst-wall ruptures, these granular masses pass out in company with the ripe spermatozoa and of course equal them in number. The cells of the cyst-wall also break up and their fragments pass into the lumen of the testis. Still another contribution to the mass of degenerating cells comes from the remains of the "connecting body" or cytoplasmic neck which for a time unites two sister spermatids and which contains the Zwischenkörper. In spite of all these sources of supply enumerated above, a difficulty still presents itself in the effort to explain all of the degenerating cells in the sperm duct. Even when we grant that they arise in three ways, viz.: from the cells of the cyst-walls, from the granular, cytoplasmic masses discarded by the spermatozoa and from the connecting-bodies and their contained mid-bodies, still the number appears to be too enormous to be wholly accounted for in these ways. No doubt there is considerable fragmentation but the size of the majority of the cytoplasmic masses is at least equal to the size of the contracted masses as they escape from the cyst (compare Figs. 56 and 59) so that fragmentation is not a satisfactory explanation, especially as the total mass of them far exceeds the total mass of the spermatozoa. It might be thought probable that the spermatozoa would pass through the ducts more rapidly than the degenerating cells and so leave a relatively large number of them behind, but it is difficult to see how this could be true since the spermatozoa are rolled up and are, in all probability, entirely inert. The contraction of the wall of the sperm duct would surely propel the degenerating cells as rapidly as the rolled up, temporarily inactive spermatozoa.

In the duct are found some cells of a type quite distinct from those already described and which could not have arisen in any of the ways mentioned. These are comparatively large, somewhat oval cells and closely resemble the rolled up spermatozoa in size and outline but differ from them in showing little or no affinity for nuclear stains. If it seemed probable that a spermatozoön would remain coiled during the process of degeneration,

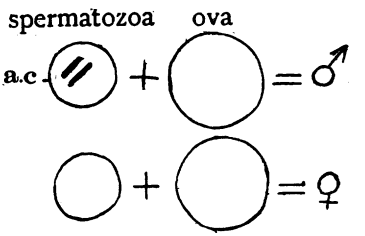
then we might suppose that these large, faintly stained cells represent degenerating spermatozoa and as they seem to be about as numerous as the deeply stained spermatozoa their presence might mean that only one half of the latter become functional. It seems more probable, however, that a spermatozoön would uncoil in the process of degeneration and it also seems likely that the chromatin in the head would retain its staining capacity for a long time. I am, therefore, at a loss to explain the presence of these large, oval cells.

Significance of the Accessory Chromosomes.

Until comparatively recent times the problem of sex-determination has been approached chiefly from the outside and much experimental work has been done in the attempt to prove that external factors, such as nutrition, temperature, etc., do influence sex. Within the last few years, however, minute cytological research has directed attention to internal factors, namely, to the nuclei of the germ-cells themselves, and there is now good reason to believe that the problem can be put on a morphological basis. McClung's ('02*b*) brilliant idea that dimorphism of the spermatozoa caused by the presence or absence of the accessory chromosomes might have a direct bearing on the determination of sex has been strongly supported by Wilson ('05*a*, *b*, *c*, '06) and Stevens ('05*b*, '06*b*) and, more recently, by Boring ('07) in their work upon insects. These authors find cases in which the somatic cells of the male contain one less chromosome than the somatic cells of the female or, in cases where one half of the spermatozoa contain a very small chromosome represented in the other half by a large chromosome, the somatic cells of the male and female show corresponding differences.

In the spider *Agalena nævia* I have shown that dimorphism of the spermatozoa obtains, one half of them having two accessory chromosomes and one half of them lacking these elements. The comparative number of chromosomes in the somatic cells of the two sexes could not be determined, but a comparison of the developing germ-cells was made with reference to the presence or absence of the accessory chromosomes. In the spireme stage of the growth period of the primary spermatocytes, the two rod-

like accessory chromosomes are more conspicuous than at any other time during the development of the sperm-cells (Fig. 13). On the other hand, a study of the primary oöcytes at a corresponding stage reveals the fact that in them no trace of the accessory chromosomes can be found (Fig. 57). It might be contended that during the much longer resting stage of the oöcytes, the accessory chromosomes dissolve into the reticulum as do the ordinary chromosomes but an argument against such an interpretation is the fact that the same lack is evident even in the extremely small ovaries of very young spiders captured in June — two months before the breeding season. At *all* stages of the growth period, in the youngest as well as in the oldest oöcytes, no accessory chromosomes can be found. Now, while this is not in itself conclusive evidence that all of the cells of the female spider lack the accessory chromosomes, it seems *probable* that such is the case in the light of the work upon insects and in view of the fact that one half of the spider spermatozoa lack these two elements. Since the primary oöcytes have no accessory chromosomes, in all probability the mature eggs lack them also. If, then, an egg be fertilized by a spermatozoön possessing two accessory chromosomes, a male would be produced but if an egg be fertilized by a spermatozoön which does not possess them, a female would be produced. This interpretation brings the spider into line with the insects in support of the view that the accessory chromosomes may be directly connected with sex-determination, the main difference between the insects and spiders being that in the former the female has the greater number of chromosomes while in the latter the male is the "favored" one.



Comparison of Results.

The earliest work on spider spermatogenesis which was done under sufficiently modern methods to concern us here is that of Wagner ('96*b*), but his complete paper, published in the Russian language, is not accessible to me. From a preliminary report in a German periodical ('96*a*) and from several short reviews, I judge that his work has been rather comprehensive, including the history of the germ-cells from the early spermatogonia to the mature spermatozoa. His studies were mainly concerned with *Agalena* and it is therefore with special interest that I compare my results with his. In the spermatogonia he states that division does not occur according to the ordinary method of karyokinesis, nor is it amitotic, so one is puzzled to know what method of division he did observe. He also makes the surprising statement that the nuclei of the spermatocytes are much smaller than the nuclei of the spermatogonia of the last generation. In the growth period and also in the primary spermatocytic division Wagner finds a peculiar nucleolus and while his description is far from accurate, I have no hesitancy in saying that under this term he describes the accessory chromosomes. This so-called "nucleolus" has a compact, elliptical form and is always peripheral in position, never lying inside the spireme threads. In the succeeding division it divides either in the plane of the equatorial plate or nearer one pole and in the latter case it is cast out into the cytoplasm (!). My results show that while the accessory chromosomes are usually in a peripheral position, they occupy a more central position in the growth period and are then surrounded by the spireme threads. As to the accessory chromosomes — they do not divide at all in the first division but are carried over bodily into one of the two daughter cells.

Early workers described the spermatozoön as of a disk-like, aberrant form showing no resemblance to the ordinary type. Wagner was the first to discover that this peculiar looking spermatozoön, with apparently no organ of locomotion, does in reality essentially agree in its development with the ordinary type and possesses head, tail and apical body. He also demonstrated clearly that the disk-like form is due to the fact that the ripe spermatozoön rolls itself up in such a way that it is difficult to

recognize its resemblance to a typical spermatozoön. In the transformation of the spermatid, he incorrectly explained the growth of the axial filament, holding that it first appears in the cytoplasm and later makes connection with the nucleus and he was also mistaken in believing that a portion of the nucleus takes no part in the formation of the spermatozoön head and later disappears. Wagner's work, on the whole, added much to our understanding of the peculiar spider spermatozoön and made a foundation for the more detailed work of Bösenberg which is reviewed below.

Montgomery's work ('05) on *Lycosa* follows in some detail the history of the spermatocytes, and as a number of my results differ from his, it seems worth while to enumerate the main points in which we disagree. A careful perusal of his text and figures, and my own observations on several different genera including *Lycosa*, lead me to believe that he has misinterpreted some points and that a further study of *Lycosa* will bring about greater harmony in our results.

1. In *Lycosa*, he says: "There is no rest stage at any period of spermatocytic history."

In *Agalena*, well-marked rest stages occur in both of the spermatocytes and in the spermatids.

2. In *Lycosa* "where the ends of two conjugated chromosomes come together is frequently found a slight notch or break which is a connecting band of linin."

In *Agalena* the point of union is marked by a greater accumulation of chromomeres at the bend of the spireme loop.

3. In *Lycosa* "the split in the prophase (of the first division) does not extend through the distal ends of the generally V-shaped loops."

In *Agalena* the split extends throughout the length of the loop.

4. In *Lycosa* the longitudinal split of the prophase of the first maturation division becomes "in some of the chromosomes a little wider than during post-synapsis but this happens with only a minority of the chromosomes in any nucleus and it is not a definitive stage in the structural change of every chromosome for the reason of its relative infrequency. Most of the chromosomes are straight or bent rods."

In *Agalena*, the widening of the longitudinal split at this stage is of universal occurrence in the ordinary chromosomes and is of first importance as a foreshadowing of the opening out of the single V-shaped chromosomes to form double V's, which is the definitive form of every ordinary chromosome. Furthermore Montgomery's own figures of the telophase show many V-shaped chromosomes and indicate that in *Lycosa*, also, the chromosomes of the metaphase are double V's. On examination of my own sections of *Lycosa*, I find this to be true.

5. In *Lycosa* "there is no intermediate cell-plate formed after the reduction division but after all other divisions."

In *Agalena*, the intermediate cell-plate is always found at this stage and is often conspicuous.

6. In *Lycosa* "the two univalent heterochromosomes conjugate side to side though their ends directed toward the distal nuclear pole are in closer touch than their opposite ends, in contrast to the behavior of the other chromosomes."

In *Agalena* the heterochromosomes unite, apparently, into a single mass and the union usually begins at the ends directed toward the central pole.

7. In *Lycosa* "the mode of division of the bivalent heterochromosomes was not positively determined" but in its formation "there is some evidence that the heterochromosome may behave like the others during the maturation mitoses, namely, that it may undergo a reductional division in the first and an equational division in the second mitosis. And we can say positively that the whole bivalent heterochromosome does not pass undivided into one of the second spermatocytes." (!)

In *Agalena* and several other genera the heterochromosomes clearly pass undivided into the secondary spermatocytes and in *Agalena*, at least, they are equationally divided in the second maturation mitosis.

Bösenberg's beautiful work ('05) on the spermatogenesis of the Arachnida is based chiefly upon a study of *Lycosa* and his observations begin with the telophase of the second maturation division, his work being largely confined to a detailed study of the transformation of the spermatid into the mature spermatozoön. Taking Wagner's results upon *Agalena* as a starting point, he

follows with utmost care the development of the spermatid nucleus, centrosome and idiozome. He, like Wagner, undoubtedly mistook the compact accessory chromosomes for a nucleolus which is conspicuous and often surrounded by a clear area in the spermatids. According to Bösenberg, this nuclear element disappears prior to the formation of the chromatin plate at one side of the spermatid nucleus. As a matter of fact, however, the accessory chromosomes ("nucleolus") form an important part of the chromatin plate in half of the spermatids, first stretching across the side of the nucleus where the chromatin granules later accumulate. Bösenberg traced the subdivision of the centrosome into proximal and distal portions. The distal centrosome migrates to the cell-periphery and from it grows out the delicate extracellular axial filament. The proximal centrosome moves over or through a portion of the nucleus and later becomes comparatively large and pear-shaped. It is then regarded as the connecting-piece, or middle-piece, the latter term being thought inappropriate in the spider spermatozoön. The apical body is derived from the idiozome vesicle and contains a filament which bears a small granule. The latter is derived from the connecting piece. My observations, so far as they have gone, indicate a close agreement between the transformation of the spermatid of *Lycosa* and that of *Agalena*.

To Berry ('06) belongs the credit of first reporting dimorphism of the spider spermatozoa although in her brief paper on *Epeira* she was not able to bring forward much data in support of this view. In the telophase of the last spermatogonial division, one chromosome appears to have no mate and is therefore regarded as the odd chromosome which persists as a single, univalent element in the rest stages, becoming longitudinally split in the spireme. In the first maturation mitosis, the odd chromosome is carried to but one pole and while it was not identified in the second maturation mitosis it is thought to divide along the line of the original longitudinal split, the resultant halves being carried to the opposite poles of the spindle. This view is supported by the fact that apparently one half of the spermatids contain a single chromatin mass while the other half do not.

Knowing by experience the difficulty of accurately counting

numerous rod-like chromosomes which often overlap one another and knowing also the possibility that all of the chromosomes may not necessarily lie in the plane of a given section and may therefore be counted twice, it seems to me unwise to place much reliance upon the finding of an apparently unmated chromosome in the telophase of the last spermatogonia. Again, the split and unsplit odd chromosome of the rest stage and of the growth period may be two univalent heterochromosomes or accessory chromosomes before and after conjugation, as in *Agalena*. In the primary spermatocytic division of *Epeira* the split, odd chromosome which is carried to but one pole is probably the two univalent accessory chromosomes and while Berry surmised that the halves of this single element are separated in the secondary spermatocytic division, it is possible that each half of the odd chromosome, or, as I believe, each univalent element of the two accessory chromosomes, splits lengthwise and is equally distributed to the opposite poles. While Berry finds a single chromatin mass in half of the spermatids, I find in *Agalena* two accessory chromosomes in half of the spermatids. These, however, eventually fuse into a single mass, so there is no real discrepancy here. Our main point of issue is with the *origin* of the accessory chromosomes. If it be granted that they originate from two spermatogonial chromosomes rather than from one, then it is possible to interpret all of Berry's figures in such a way that the odd chromosome of *Epeira* and the two accessory chromosomes of *Agalena* will be seen to be nearly identical in behavior and fate. The forms of the ordinary chromosomes of *Epeira* in the prophase and metaphase of the first maturation division are somewhat obscure in the figures but are described as V's, rings, rods and crosses. Now one who is familiar with the double V-shaped chromosomes in other forms can readily see how they might appear as represented, especially if they are overstained or closely packed together. Furthermore, my own sections of *Epeira* show plainly that the definitive form of the chromosomes in the equatorial plate of the first maturation division is that of a double V.

It may not be out of place to mention here the two chief errors in my own previous work ('05). In the first place I failed to

find the division of the accessory chromosomes in the second maturation mitosis and in the second place I failed to find the tail of the spermatozoön. These points have been fully discussed in the body of this paper.

Although I am fully aware that it is easy to read one's own interpretation into the work of others, nevertheless I am confident that the spermatogenesis of at least three genera of spiders — *Agalena*, *Epeira* and *Lycosa* — will be found to agree in all essential points.

Summary.

1. In the spermatogonia the nuclei are unusually large. The chromosomes are rod-like and are probably at least fifty-two in number. Two of them appear different from the others and are regarded as the accessory chromosomes.

2. In the primary spermatocytes, the ordinary chromosomes conjugate end to end in synapsis to form V-shaped chromosomes These open out along the line of the longitudinal split of the spireme to form double V's and divide reductionally. In the early rest stage the two accessory chromosomes take the form of two chromatin nucleoli which later unite into a single chromatin nucleolus. At the beginning of the growth period they again take the form of two rods which later conjugate side to side during a small fraction of the growth period. In mitosis they pass over bodily into but one of the two daughter cells.

3. In the second spermatocytic division the V-shaped chromosomes and also the two rod-like accessory chromosomes divide equationally. The reduced number of the ordinary chromosomes is probably at least twenty-five.

4. The spermatozoön has a well-developed axial filament derived from the distal centrosome. The proximal centrosome gives rise to the end-knob.

5. There is dimorphism of the spermatozoa, half of them containing two accessory chromosomes and half of them lacking these elements.

6. Since the accessory chromosomes are more conspicuous during the growth period of the primary spermatocytes than at any other time and in the primary oöcytes no trace of them can be found and since the dimorphism of the spermatozoa is due to

the presence or absence of these peculiar elements, it seems probable that an egg fertilized by a spermatozoön possessing the accessory chromosomes develops into a male while an egg fertilized by a spermatozoön which lacks the accessory chromosomes develops into a female. The results of my work upon the spider, therefore, furnish further evidence in support of McClung's theory of sex-determination.

7. Degenerating cells or cell fragments which envelop the ripe spermatozoa in the sperm-ducts come from at least four different sources. These are as follows: (*a*) Broken down walls of empty cysts, (*b*) cell bodies from which the ripe spermatozoa have escaped, (*c*) "connecting bodies" of sister spermatids, and their contained mid-bodies, (*d*) large, oval cells which resemble the rolled up spermatozoa, in size and outline.

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May, 1908.

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EXPLANATION OF PLATES.

All figures were drawn with the aid of a camera lucida under Bausch and Lomb one twelfth oil immersion and Zeiss Comp. oc. 12. at table level and in the plates are reduced about one eighth.

All not otherwise specified are taken from *Agalena nævia*.

Abbreviations: *a.c*, accessory chromosome; *a*, mature spermatozoön; *b*, degenerating cytoplasmic body; *c*, degenerating spermatid; *d*, degenerating spermatozoön (?); *y*, yolk nucleus.

EXPLANATION OF PLATE I.

FIGS. 1, 2. Spermatogonium. Metaphase.

FIGS. 3, 4. Spermatogonium. Anaphase.

FIGS. 5–7. Primary spermatocytes. Rest stage.

FIGS. 8, 9. Primary spermatocytes. Contraction stage.

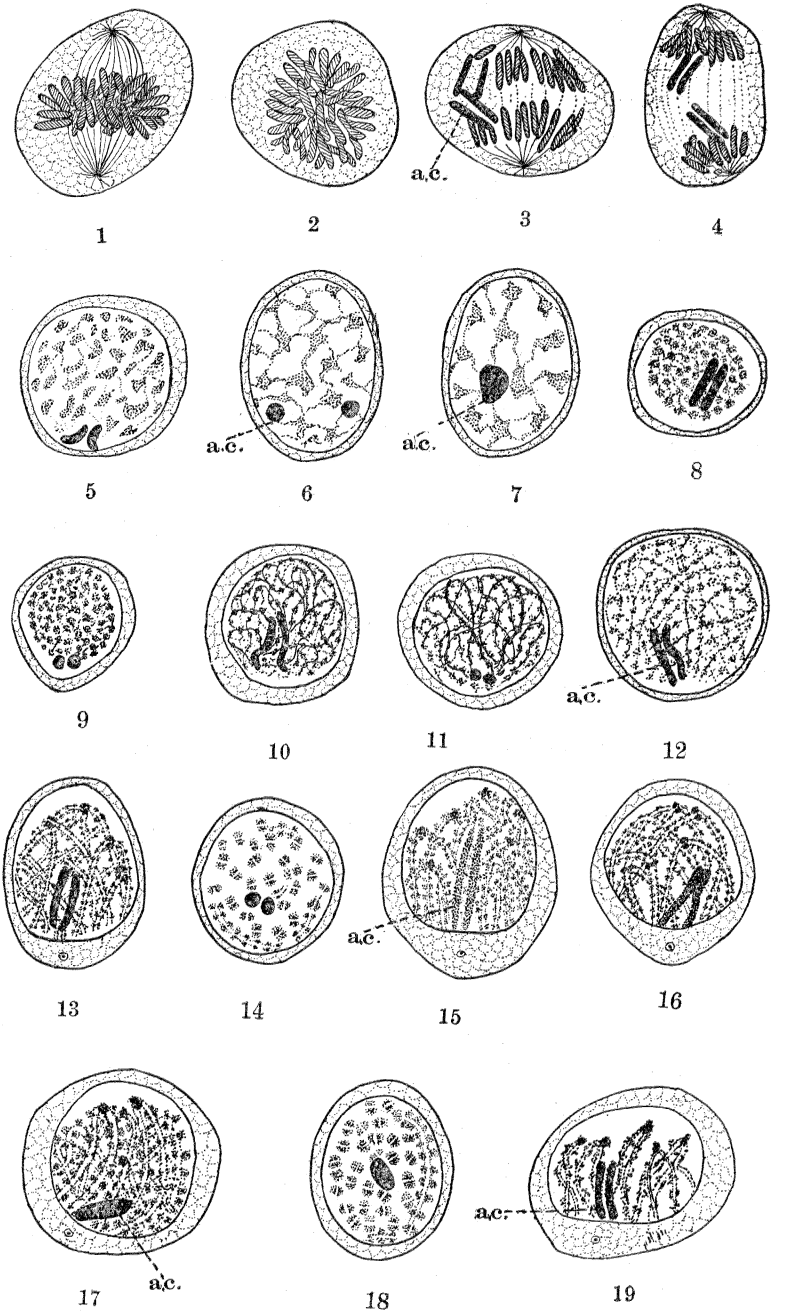
FIGS. 10–12. Primary spermatocytes of growth period. Spireme not split.

FIG. 13. Primary spermatocyte. Spireme thread longitudinally split.

FIG. 14. Primary spermatocyte. Cross-section of split spireme.

FIGS. 15–17. Primary spermatocytes. Split in spireme thread widens. The accessory chromosomes conjugate.

FIG. 18. Primary spermatocyte. Cross-section of split spireme and conjugated accessory chromosomes.


a.c.
a.c.
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Explanation of Plate II.

Figs. 19–21. Primary spermatocytes. The spireme loops contract. The accessory chromosomes separate again.

Figs. 22, 23. Primary spermatocytes. Prophase. Single V-shaped chromosomes open out into double V's.

Fig. 24. Primary spermatocyte. Metaphase.

Fig. 25. Primary spermatocyte. Division of double V's to form single V's. Accessory chromosomes passing to one pole.

Fig. 26. Primary spermatocyte. Anaphase.

Fig. 27. Primary spermatocyte. Anaphase. Pole view, *a.c.* split.

Fig. 28. Primary spermatocyte. Telophase.

Figs. 29, 30. Secondary spermatocytes. Rest stage.

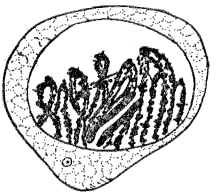
Fig. 31. Secondary spermatocyte. Prophase. Accessory chromosomes show precocious, longitudinal split.

Figs. 32, 33. Secondary spermatocytes. Metaphase.

Fig. 34. Secondary spermatocyte. Division of V-shaped chromosomes into rod-shaped chromosomes.

Figs. 35, 36. Secondary spermatocytes. Anaphase. Accessory chromosomes divide equationally.

Fig. 37. Secondary spermatocyte. Late anaphase, showing twenty-five rod-shaped chromosomes.



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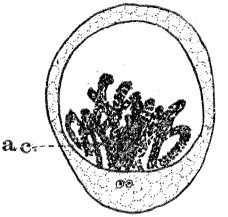

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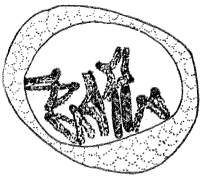

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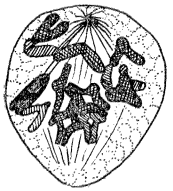

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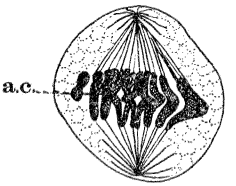

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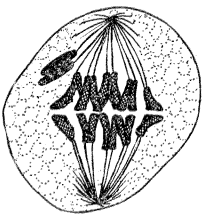

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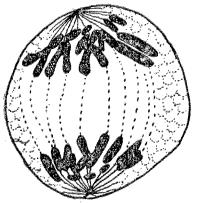

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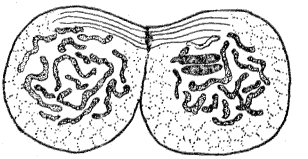

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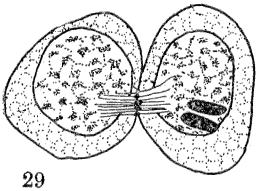

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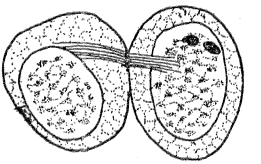

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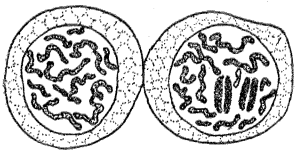

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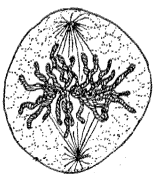

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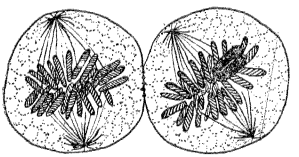

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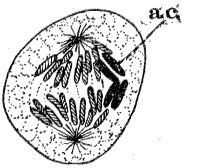

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Explanation of Plate III.

Figs. 38, 39. Secondary spermatocytes. Late anaphase.

Figs. 40, 41. Secondary spermatocytes. Telophase. Accessory chromosomes large and conspicuous.

Figs. 42, 43. Spermatids. Axial filament grows out from centrosome.

Figs. 44, 45. Spermatids. Interzonal fibers disappear. The mid-body persists at center of "connecting-body."

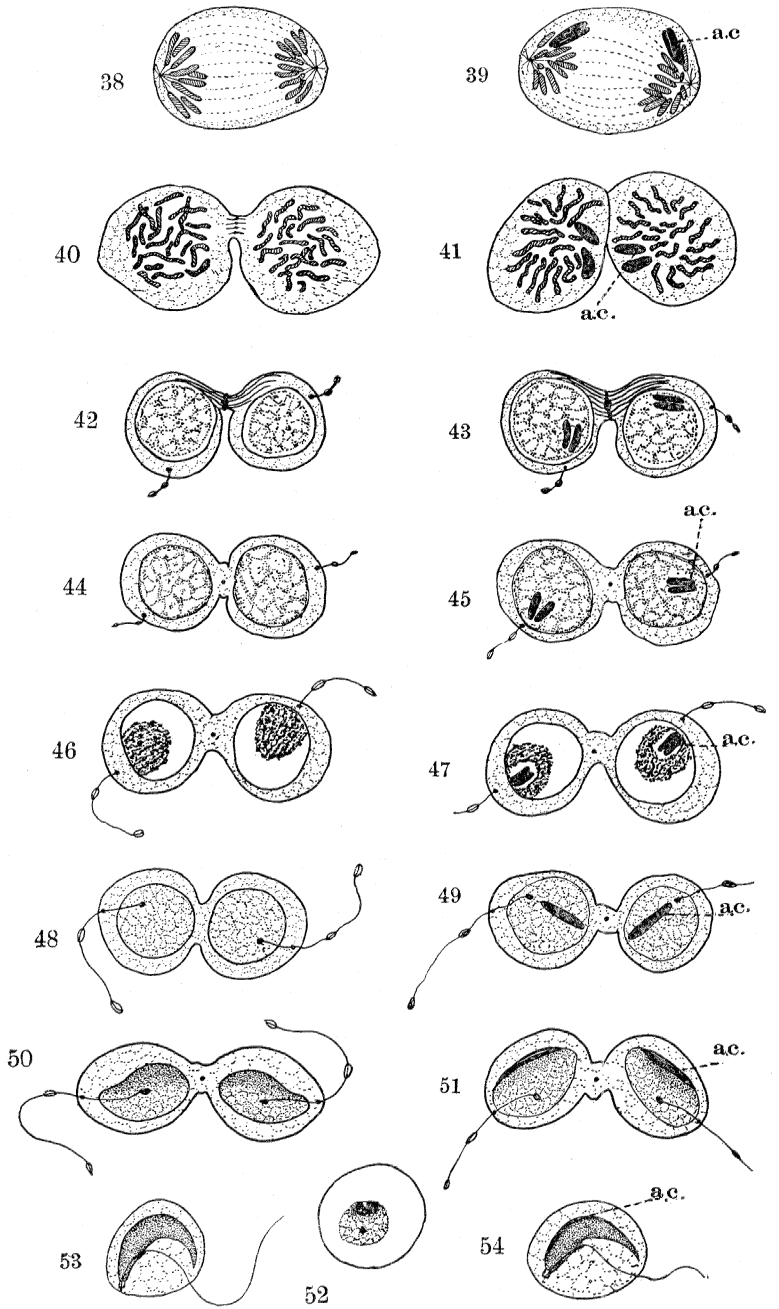
Figs. 46, 47. Spermatids. Contraction stage. Accessory chromosomes surrounded by clear area.

Figs. 48, 49. Spermatids. Accessory chromosomes unite and pass toward central pole; proximal centrosome passes over or into nucleus. Axial filament lengthens.

Figs. 50, 51. Spermatids. Nucleus elongated. Accessory chromosomes again show partial separation. Chromatin plate forms. Proximal centrosomes becomes larger.

Fig. 52. Spermatid. Cross-section of accessory chromosomes. Chromatin plate and proximal centrosome.

Figs. 53, 54. Spermatozoa. Apical granule projects from apical body. Proximal centrosome becomes end-knob. Accessory chromosomes on convex side of head in Fig. 53.


38
39
a.c
40
41
a.c.
42
43
44
45
a.c.
46
47
a.c.
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49
a.c.
50
51
a.c.
53
52
54
a.c.

Explanation of Plate IV.

Fig. 55. Spermatozoa escaped from cell bodies.

Fig. 56. Spermatozoa contracted and coiling up. Cell bodies contracting also.

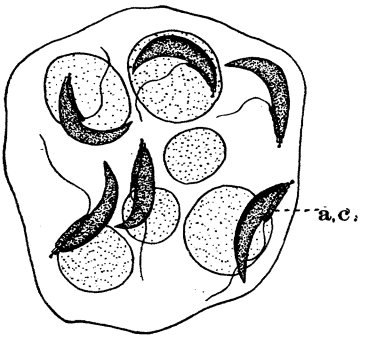
Fig. 57. Primary oöcyte, growth period.

Fig. 58. Cell bodies. Stages in degeneration, *a–e*.

Fig. 59. Portion of sperm-duct in breeding season.

Fig. 60. Cyst full of degenerating spermatids. (*Pholcus phalangioides*.)

Fig. 61. (*a*) Mature spermatozoa, (*b*) degenerating cell bodies and (*c*) degenerating spermatids found in lumen of testis of *Pholcus phalangioides*.



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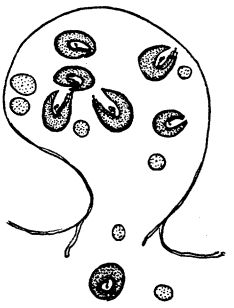

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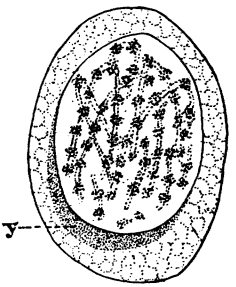

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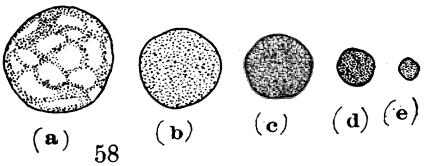

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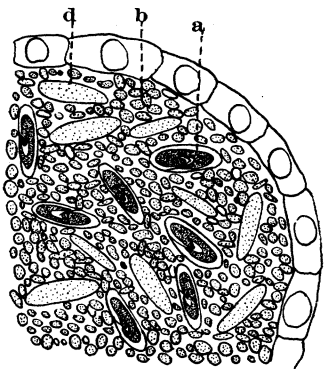

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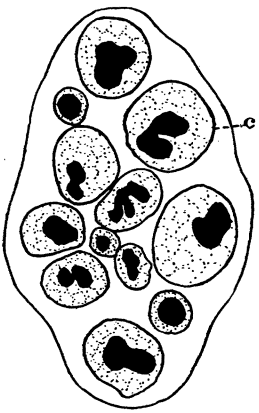

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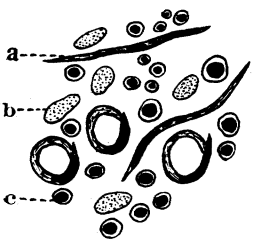

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